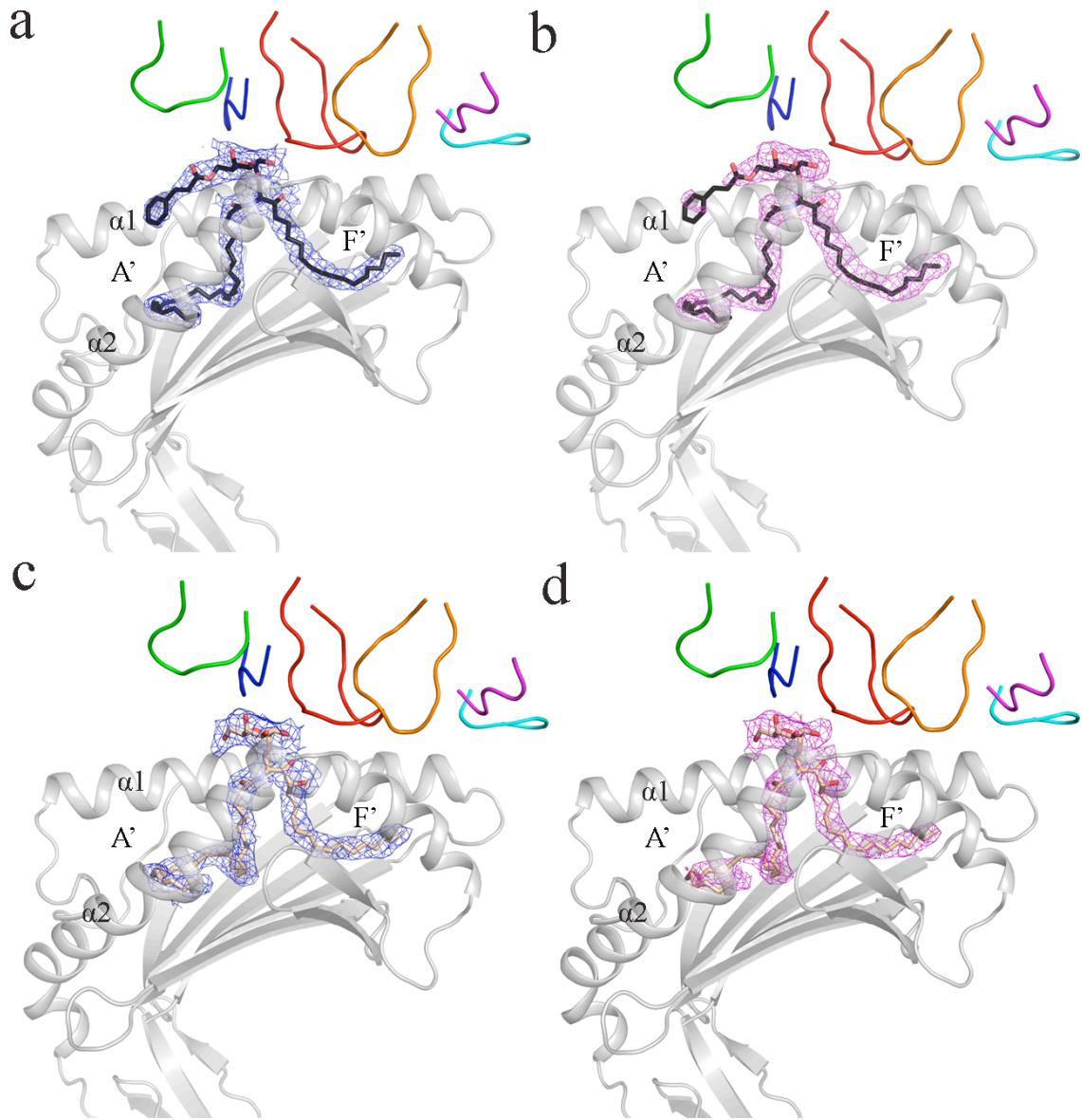


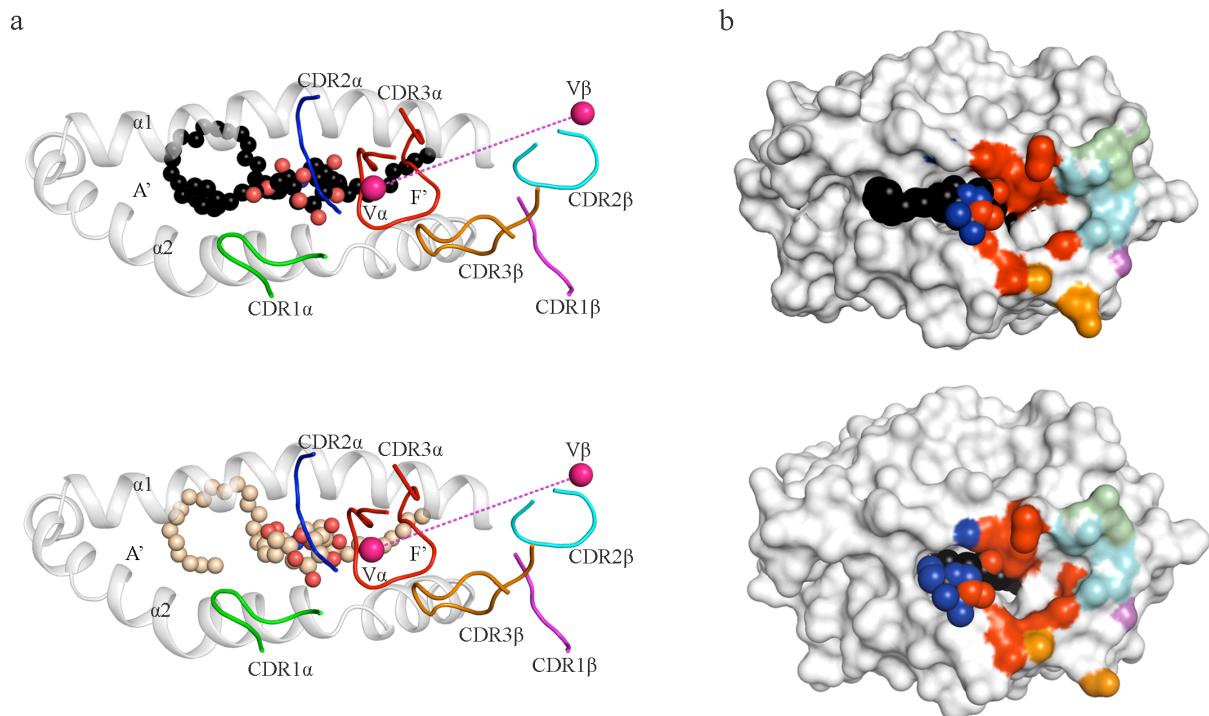
## **Supplemental Information**

### **Dual Modifications of an $\alpha$ -Galactosyl Ceramide Synergize to Promote Activation of Human Invariant Natural Killer T Cells and Stimulate Anti-Tumor Immunity**

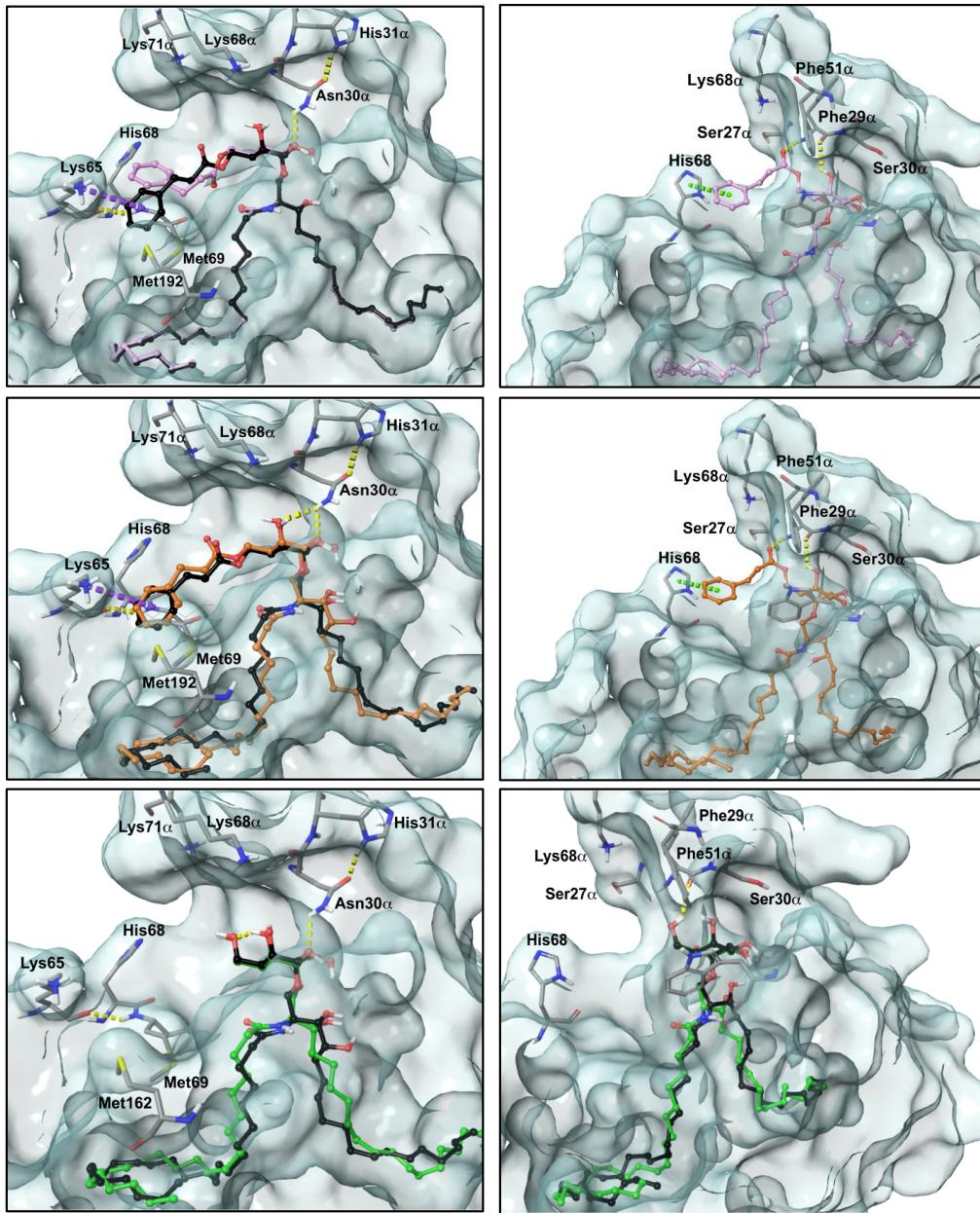
**Divya Chennamadhavuni, Noemi Alejandra Saavedra-Avila, Leandro J. Carreño, Matthew J. Guberman-Pfeffer, Pooja Arora, Tang Yongqing, Hui-Fern Koay, Dale I. Godfrey, Santosh Keshipeddy, Stewart K. Richardson, Srinivasan Sundararaj, Jae Ho Lo, Xiangshu Wen, José A Gascón, Weiming Yuan, Jamie Rossjohn, Jérôme Le Nours, Steven A. Porcelli, and Amy R. Howell**



**Figure S1 related to Figure 5.** **a.** 2Fo-Fc electron density map (in blue) at  $0.8\sigma$  level of AH10-7 (in black sticks). **b.** Fo-Fc electron density map (in light magenta) at  $2.2\sigma$  level of AH10-7. **c.** 2Fo-Fc electron density map (in blue) at  $0.8\sigma$  level of KRN7000 (in wheat sticks). **d.** Fo-Fc electron density map (in light magenta) at  $2.2\sigma$  level of KRN7000. The mouse CD1d and the 2C12 TCR CDR loops are shown as cartoon representations: mCD1d, grey; CDR1 $\alpha$ , blue; CDR2 $\alpha$ , green; CDR3 $\alpha$ , red; CDR1 $\beta$ , magenta; CDR2 $\beta$ , cyan; CDR3 $\beta$ , orange.



**Figure S2 related to Figure 5. a.** Top view of the mCD1d binding cleft with the bound AH10-7 (top panel) and KRN7000 (bottom panel) The mouse CD1d and the 2C12 TCR CDR loops are shown as cartoon representations: mCD1d, grey; CDR1 $\alpha$ , blue; CDR2 $\alpha$ , green; CDR3 $\alpha$ , red; CDR1 $\beta$ , magenta; CDR2 $\beta$ , cyan; CDR3 $\beta$ , orange. AH10-7 and KRN7000 are shown as black and wheat spheres, respectively. The center of mass positions of the  $\alpha$ - and  $\beta$ -variable domains of the 2C12 TCR are shown as hotpink spheres. **b.** Footprint of the 2C12 TCR onto the mCD1d-antigen molecular surface in 2C12 TCR-mCD1d-AH10-7 (top panel) and 2C12 TCR-mCD1d-KRN7000 (bottom panel) ternary complexes. Molecular surface of mCD1d; light grey; the 2C12 TCR CDR loops are colored as in (a). AH10-7 and KRN7000 are shown as spheres.



**Figure S3 related to Figure 7.** Best ranked docking poses for AH10-7 (lavender), AH15-1 (orange) and AH03-1 (green) into the m- (left) and hCD1d-TCR (right) complexes. The crystallographic pose of AH10-7 is shown in black. H-bonding,  $\pi-\pi$  stacking, and  $\pi$ -cation interactions are indicated by yellow, green and purple dashed lines respectively. The m- and hCD1d-TCR complexes were prepared from PDB 6BNL and 3VWK using Maestro version 10.6.014.

	<b>2C12 TCR-mCD1d-AH10-7</b>	<b>2C12 TCR-mCD1d-KRN7000</b>
<b>Data collection</b>		
Temperature	100K	100K
Resolution limits (Å)	83.01-2.60 (2.74-2.60)	44.45-3.2 (3.33-3.2)
Space Group	P12 <sub>1</sub> 1	P12 <sub>1</sub> 1
Cell dimensions (Å)	$a=79.7, b=150.3, c=100.1$ $\alpha=\gamma=90.00^\circ \beta=96.1^\circ$	$a=79.9, b=150.5,$ $c=101.2$ $\alpha=\gamma=90.00^\circ \beta=95.2^\circ$
Total N° observations	290803 (43464)	132553 (15332)
N° unique observations	71925 (10545)	39296(4451)
Multiplicity	4.0 (4.1)	3.4 (3.4)
Data completeness	99.9 (100.0)	99.7 (99.9)
Wilson B-factors (Å <sup>2</sup> )	66.9	57.2
I/σ <sub>1</sub>	21.2 (2.3)	4.8 (2.2)
R <sub>p.i.m</sub> <sup>1</sup> (%)	4.9 (37.5)	10.2 (30)
<b>Refinement statistics</b>		
R <sub>factor</sub> <sup>2</sup> (%)	18.3	20.2
R <sub>free</sub> <sup>3</sup> (%)	21.3	22
Non hydrogen atoms		
- Protein	12184	12192
- Water	172	102
- Heterogen	250	232
Ramachandran plot (%)	99.9	99.9
- Allowed region	0.1	0.1
- Disallowed region		
rmsd bonds (Å)	0.010	0.007
rmsd angles (°)	1.12	0.90

<sup>1</sup> R<sub>p.i.m</sub> =  $\sum_{hkl} [1/(N-1)]^{1/2} \sum_i |I_{hkl,i} - \langle I_{hkl} \rangle| / \sum_{hkl} \langle I_{hkl} \rangle$

<sup>2</sup> R<sub>factor</sub> = (  $\sum |F_o - |F_c|| / (\sum |F_o|)$  ) - for all data except as indicated in footnote 3.

<sup>3</sup> 5% of data was used for the R<sub>free</sub> calculation

Values in parentheses refer to the highest resolution bin.

**Table S1 Related to Figure 5.** Data collection and refinement statistics.

<b>TCR gene</b>	<b>TCR residues</b>	<b>CD1d residues</b>	<b>Bond type</b>
CDR1 $\alpha$	Pro28	Val72, Ser76	VDW
CDR3 $\alpha$	Asp94	Arg79	VDW
CDR3 $\alpha$	Asp94-O $\delta$ 2	Arg79-N $\eta$ 1, Arg79-N $\eta$ 2	HB
CDR3 $\alpha$	Arg95	Ser76, Arg79, Asp80	VDW
CDR3 $\alpha$	Arg95-N $\epsilon$ 1	Ser76-O	HB
CDR3 $\alpha$	Arg95-N $\epsilon$ 1	Asp80-O $\delta$ 1	HB
CDR3 $\alpha$	Gly96	Ala152, Asp153	VDW
CDR3 $\alpha$	Ser97	Val149, Ala152	VDW
CDR3 $\alpha$	Leu99	Arg79, Asp80, Glu83, Leu84, Met87, Val149	VDW
CDR3 $\alpha$	Gly100	Arg79	VDW
CDR3 $\alpha$	Arg103	Arg79, Glu83	VDW
CDR2 $\beta$	Tyr50	Glu83, Lys86, Met87	VDW
CDR2 $\beta$	Tyr50-OH	Glu83-O $\epsilon$ 2	HB
CDR3 $\beta$	Glu97	Lys148, Ala152	VDW
FW $\beta$	Tyr48	Glu83, Lys86	VDW
FW $\beta$	Tyr48-OH	Glu83-O $\epsilon$ 1, Glu83-O $\epsilon$ 2, Lys86-N $\zeta$ Arg21, Lys86	HB
FW $\beta$	Glu56		VDW
FW $\beta$	Glu56-O $\epsilon$ 1	Arg21-N $\eta$ 1	HB
<b>TCR gene</b>	<b>TCR residues</b>	<b>AH10-7 atoms</b>	<b>Bond type</b>
CDR1 $\alpha$	Pro28	C1, O5	VDW
CDR1 $\alpha$	Asn30	3', 4'-OH, C2, C3	VDW
CDR1 $\alpha$	Asn30-O $\delta$ 1	3'-OH	HB
CDR1 $\alpha$	Asn30	3'-OH, 4'-OH	VDW
CDR3 $\alpha$	Arg95	2'-OH, 3'-OH, C2	VDW
CDR3 $\alpha$	Gly96	C2	VDW
CDR3 $\alpha$	Gly96-N	2'-OH	HB

HB: Hydrogen bond, VDW: van der Waals. Cut-off at 4 Å for VDW interactions and 3.5 Å for HB.

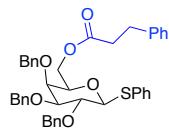
**Table S2 related to Figure 5.** 2C12 TCR contacts with AH10-7 and mCD1d.

<b>TCR gene</b>	<b>TCR residues</b>	<b>CD1d residues</b>	<b>Bond type</b>
CDR1 $\alpha$	Pro28	Ser76	VDW
CDR3 $\alpha$	Asp94	Arg79	VDW
CDR3 $\alpha$	Asp94-O $\delta$ 1	Arg79-N $\eta$ 2	HB
CDR3 $\alpha$	Asp94-O $\delta$ 2	Arg79-N $\eta$ 1	HB
CDR3 $\alpha$	Arg95	Ser76, Arg79, Asp80	VDW
CDR3 $\alpha$	Arg95-N $\eta$ 1	Ser76-O $\gamma$	HB
CDR3 $\alpha$	Arg95-N $\varepsilon$	Asp80-O $\delta$ 1	HB
CDR3 $\alpha$	Arg95-N $\eta$ 1	Asp80-O $\delta$ 1	HB
CDR3 $\alpha$	Gly96	Ala152, Asp153	VDW
CDR3 $\alpha$	Ser97	Val149, Ala152	VDW
CDR3 $\alpha$	Leu99-O	Arg79-N $\eta$ 2	HB
CDR3 $\alpha$	Leu99	Arg79, Asp80, Leu84, Met87, Val149	VDW
CDR3 $\alpha$	Gly100	Arg79	VDW
CDR3 $\alpha$	Arg103	Arg79	VDW
CDR2 $\beta$	Tyr50	Glu83, Lys86, Met87	VDW
CDR2 $\beta$	Tyr50-OH	Glu83- O $\varepsilon$ 2	HB
CDR3 $\beta$	Glu97	Lys148, Ala152	VDW
FW $\beta$	Tyr48	Glu83, Lys86	VDW
FW $\beta$	Tyr48-OH	Glu83-O $\varepsilon$ 2, Lys86-N $\zeta$	HB
FW $\beta$	Glu56	Lys86	VDW
<b>TCR gene</b>	<b>TCR residues</b>	<b>AH10-7 atoms</b>	<b>Bond type</b>
CDR1 $\alpha$	Pro28	O6, C1, C6	VDW
CDR1 $\alpha$	Asn30	4'-OH, C2, C3	VDW
CDR1 $\alpha$	Asn30-N $\delta$ 2	3'-OH	HB
CDR1 $\alpha$	Asn30	3'-OH, 4'-OH	VDW
CDR3 $\alpha$	Asp94	C1	VDW
CDR3 $\alpha$	Arg95	2'-OH, 3'-OH, C2	VDW
CDR3 $\alpha$	Arg95-N $\varepsilon$	O3	HB
CDR3 $\alpha$	Gly96	C2, 2'-OH	VDW
CDR3 $\alpha$	Gly96-N	2'-OH	HB

HB: Hydrogen bond, VDW: van der Waals. Cut-off at 4 Å for VDW interactions and 3.5 Å for HB.

**Table S3 related to Figure 5.** 2C12 TCR contacts with KRN7000 and mCD1d.

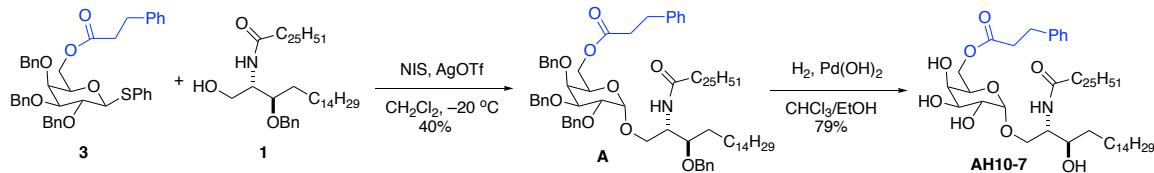
## Synthesis and characterization of AH10-7 and AH15-1.



### Phenyl 2,3,4-tri-O-benzyl-6-hydrocinnamoyl-1-thio- $\beta$ -D-galactopyranoside (3)

Hydrocinnamic acid (0.59 g, 3.9 mmol), DCC (0.76 g, 3.7 mmol) and *N,N*-Dimethylaminopyridine (DMAP) (0.087 g, 0.71 mmol) were added to a solution of phenyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -D-galactopyranoside<sup>1</sup> (1.93 g, 3.56 mmol) in DCM (20 mL). The solution was then stirred at rt for 24 h. The resulting reaction mixture was concentrated and purified by flash chromatography on silica gel (petroleum ether/EtOAc, 85:15) to afford **3** as a white solid (1.47 g, 61%): mp 66–68 °C;  $[\alpha]^{22}_D$  -23.2 (c 1.78, DCM); IR (neat) 3026, 2926, 2864, 1721, 1452, 733 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.65–7.62 (m, 2H), 7.47–7.23 (m, 23H), 5.03 (d, *J* = 11.5 Hz, 1H), 4.89 (d, *J* = 10.2 Hz, 1H), 4.86–4.78 (m, 3H), 4.67 (d, *J* = 9.6 Hz, 1H), 4.65 (d, *J* = 11.5 Hz, 1H), 4.34 (dd, *J* = 11.2, 6.9 Hz, 1H), 4.20 (dd, *J* = 11.2, 5.6 Hz, 1H), 4.01 (dd, *J* = 9.4, 9.4 Hz, 1H), 3.83 (d, *J* = 2.0 Hz, 1H), 3.63 (dd, *J* = 9.2, 2.5 Hz, 1H), 3.58 (dd, *J* = 6.2, 6.2 Hz, 1H), 2.97 (t, *J* = 7.7 Hz, 2H), 2.64 (t, *J* = 7.9 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 172.5, 140.4, 138.4, 138.4, 138.3, 134.1, 131.9, 128.9, 128.6, 128.5, 128.4, 128.4, 128.2, 127.9, 127.8, 127.7, 127.4, 126.5, 87.9, 84.3, 77.5, 76.2, 75.8, 74.4, 73.6, 73.3, 63.5, 35.7, 30.9; HRMS (ESI) calculated for C<sub>42</sub>H<sub>46</sub>NO<sub>6</sub>S (M + NH<sub>4</sub>)<sup>+</sup> *m/z*: 692.3046, found: 692.3037.

### Scheme 1. Synthesis of AH10-7



**(2*S*,3*R*)-3-O-Benzyl-1-O-(2,3,4-tri-O-benzyl-6-hydrocinnamoyl- $\alpha$ -D-galactopyranosyl)-2-(*N*-hexacosanoylamino)octadecan-1,3-diol (**A**)**

Phenyl 2,3,4-tri-O-benzyl-6-hydrocinnamoyl-1-thio- $\beta$ -D-galactopyranoside (**3**) (100 mg, 0.148 mmol) and (2*S*,3*R*)-3-O-benzyl-2-(*N*-hexacosanoylamino)octadecan-1,3-diol (**1**)<sup>2</sup> (137 mg, 0.177 mmol) were dried by azeotroping with toluene (3 X 10 mL). The mixture was dissolved in DCM (4 mL), and freshly ground 4 Å MS (50 mg) were added. The mixture was cooled to -20 °C, and *N*-iodosuccinimide (43.0 mg, 0.192 mmol) and silver triflate (12.6 mg, 0.0488 mmol) were added. The solution was stirred at -20 °C until the donor was consumed (based on TLC). The mixture was then stirred an additional 10–20 min at -20 °C, followed by the addition of Et<sub>3</sub>N (2 mL). The reaction mixture was diluted with DCM (20 mL) and filtered through Celite. The filtrate was concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded **A** as a sticky solid (79 mg, 40%): [ $\alpha$ ]<sup>21</sup><sub>D</sub> +28.9 (c 2.97, DCM); IR (neat) 3305, 2917, 2849, 1730, 1639, 1467, 1053, 695 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.43–7.14 (m, 25H), 5.80 (d, *J* = 8.7 Hz, 1H), 4.97 (d, *J* = 11.4 Hz, 1H), 4.91–4.73 (m, 4H), 4.67 (d, *J* = 11.8 Hz, 1H), 4.59 (d, *J* = 11.4 Hz, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.46 (d, *J* = 11.6 Hz, 1H), 4.28–4.22 (m, 1H), 4.17 (dd, *J* = 11.2, 6.9 Hz, 1H), 4.09–4.05 (m, 2H), 3.92–3.87 (m, 2H), 3.82 (m, 1H), 3.79–3.72 (m, 2H), 3.59 (ddd, *J* = 11.5, 5.8, 5.8 Hz, 1H), 2.92 (t, *J* = 7.8 Hz, 2H), 2.58 (t, *J* = 8.4 Hz, 2H), 2.09–1.95 (m, 2H), 1.57–1.54 (m, 4H), 1.28 (m, 70H), 0.92 (t, *J*

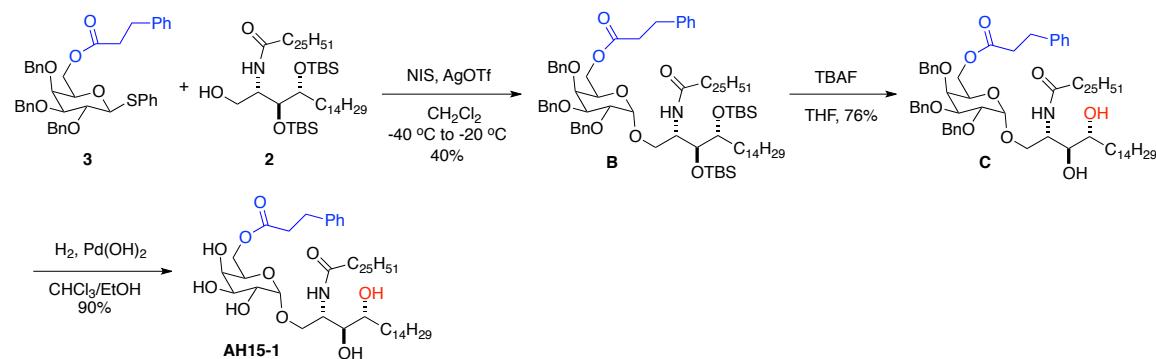
= 6.5 Hz, 3H), 0.92 (t,  $J$  = 6.5 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.9, 172.5, 140.5, 138.8, 138.6, 138.4, 128.7, 128.6, 128.6, 128.5, 128.4, 128.0, 128.0, 127.9, 127.8, 127.6, 127.6, 126.5, 99.1, 79.1, 78.9, 76.8, 74.8, 73.6, 73.5, 72.3, 69.1, 67.8, 63.6, 51.5, 37.0, 35.7, 32.1, 31.1, 31.0, 30.1, 29.9, 29.8, 29.7, 29.6, 25.9, 25.5, 22.9, 14.3; HRMS (ESI) calculated for  $\text{C}_{87}\text{H}_{132}\text{NO}_9$  ( $\text{M} + \text{H}$ ) $^+$   $m/z$ : 1334.9902, found 1334.9917.

**(2*S*,3*R*)-1-O-(6-Hydrocinnamoyl- $\alpha$ -D-galactopyranosyl)-2-(*N*-hexacosanoylamino)-octadecane-1,3-diol (AH10-7)**

(2*S*,3*R*)-3-O-Benzyl-1-O-(2,3,4-tri-O-benzyl-6-hydrocinnamoyl- $\alpha$ -D-galactopyranosyl)-2-(*N*-hexacosanoylamino)octadecan-1,3-diol (**A**) (14 mg, 0.010 mmol) was dissolved in a 1:1 mixture of EtOH (3.1 mL) and  $\text{CHCl}_3$  (3.1 mL).  $\text{Pd(OH)}_2$  (20% on carbon, 50.0 mg) was added, and stirring was continued vigorously for 24 h under  $\text{H}_2$ . The mixture was filtered through Celite, and the filter cake was washed with  $\text{CHCl}_3/\text{MeOH}$  (9:1). The filtrate was concentrated, and the residue was purified by gravity chromatography on silica gel (DCM/MeOH, 100:0 to 90:10) to give **AH10-7** as a white solid (8 mg, 79%): mp 123 °C;  $[\alpha]^{22}_D -0.38$  ( $c$  1.0,  $\text{CHCl}_3/\text{MeOH}$ , 9:1); IR (KBr) 3433, 2920, 1728, 1644  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{OD}$ , 80:20)  $\delta$  7.09–7.06 (m, 2H), 7.02–6.97 (m, 3H), 4.65 (d,  $J$  = 3.6 Hz, 1H), 4.05–4.03 (m, 2H), 3.74–3.69 (m, 2H), 3.63–3.45 (m, 5H), 3.41–3.38 (m, 1H), 2.74 (t,  $J$  = 7.6 Hz, 2H), 2.46 (t,  $J$  = 8.0 Hz, 2H), 2.01 (t,  $J$  = 7.5 Hz, 2H), 1.42 (m, 2H), 1.31–1.19 (m, 2H), 1.06 (m, 70H), 0.68 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3/\text{CD}_3\text{OD}$ , 80:20)  $\delta$  174.3, 173.0, 140.1, 128.2, 128.0, 126.1, 99.4, 70.7, 69.7, 69.1, 68.7, 68.3, 67.1, 63.6, 53.3, 36.2, 35.5, 33.8, 31.7, 30.6, 29.4, 29.4, 29.2, 29.1, 25.7, 25.6,

22.4, 13.6; HRMS (FAB) calculated for  $C_{59}H_{108}NO_9$  ( $M + H$ )<sup>+</sup> *m/z*: 974.8019, found 974.8021.

**Scheme 2. Synthesis of AH15-1**



**(2*S*,3*S*,4*R*)-3,4-Di-*O*-*tert*-butyldimethylsilyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-hydrocinnamoyl- $\alpha$ -D-galactopyranosyl)-2-(*N*-hexacosanoylamino)octadecan-1,3,4-triol (B)**

Phenyl 2,3,4-tri-*O*-benzyl-6-hydrocinnamoyl-1-thio- $\beta$ -D-galactopyranoside (**3**) (124 mg, 0.184 mmol) and (2*S*,3*S*,4*R*)-3,4-di-*O*-*tert*-butyldimethylsilyl-2-(*N*-hexacosanoylamino)-octadecan-1,3,4-triol (**2**)<sup>3</sup> (342 mg, 0.369 mmol) were dried by azeotroping with toluene (3 X 10 mL). The mixture was dissolved in DCM (3.0 mL), and freshly ground 4 Å MS (50 mg) were added. The mixture was cooled to -40 °C, and *N*-iodosuccinimide (52 mg, 0.23 mmol) and silver triflate (15 mg, 0.059 mmol) were added. The mixture was stirred at -20 °C until the donor was consumed (based on TLC). The mixture was then stirred an additional 10–20 min at -20 °C, followed by the addition of  $\text{Et}_3\text{N}$  (3 mL). The reaction mixture was diluted with DCM (20 mL) and filtered through Celite. The filtrate was concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded **B** as a viscous oil (51 mg, 40%):  $[\alpha]^{22}_{\text{D}} -82.9$  (*c* 0.5,

DCM); IR (neat) 2922, 2852, 1741, 1680, 1047, 832 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.42–7.19 (m, 20H), 5.87 (d, J = 7.0 Hz, 1H), 4.94 (d, J = 11.4 Hz, 1H), 4.86 (d, J = 3.5 Hz, 1H), 4.84 (d, J = 11.8 Hz, 1H), 4.82 (d, J = 11.7 Hz, 1H), 4.75 (d, J = 11.8 Hz, 1H), 4.68 (d, J = 11.8 Hz, 1H), 4.55 (d, J = 11.4 Hz, 1H), 4.22–4.17 (m, 1H), 4.14–4.12 (m, 2H), 4.07 (dd, J = 10.1, 3.5 Hz, 1H), 4.04 (dd, J = 9.7, 3.6 Hz, 1H), 3.92–3.87 (m, 3H), 3.82 (m, 1H), 3.71–3.67 (m, 2H), 2.92 (t, J = 7.7 Hz, 2H), 2.55 (m, 2H), 2.01 (t, J = 7.5 Hz, 2H), 1.64–1.45 (m, 4H), 1.28 (m, 68H), 0.93 (s, 9H), 0.91 (s, 9H), 0.89 (m, 6H), 0.09 (s, 3H), 0.07 (s, 3H), 0.06 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 173.3, 172.3, 140.6, 138.8, 138.7, 138.5, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.1, 127.9, 127.9, 127.8, 127.6, 126.5, 99.8, 79.3, 76.7, 76.1, 76.0, 74.8, 74.6, 73.8, 73.4, 68.9, 68.7, 62.8, 51.7, 37.0, 35.7, 33.7, 32.1, 30.9, 30.1, 29.9, 29.8, 29.7, 29.6, 26.3, 26.3, 26.2, 25.9, 22.9, 18.5, 18.4, 14.3, -3.5, -3.7, -4.4, -4.6; HRMS (ESI) calculated for C<sub>92</sub>H<sub>154</sub>NO<sub>10</sub>Si<sub>2</sub> (M + H)<sup>+</sup> m/z: 1489.1111, found: 1489.1168.

**(2S,3S,4R)-1-O-(2,3,4-Tri-O-benzyl-6-hydrocinnamoyl-α-D-galactopyranosyl)-2-(N-hexacosanoylamino)octadecan-1,3,4-triol (C)**

Tetrabutylammonium fluoride (TBAF) (1 M in THF, 0.14 mL, 0.14 mmol) was added to a stirred solution of (2S,3S,4R)-3,4-di-O-*tert*-butyldimethylsilyl-1-O-(2,3,4-tri-O-benzyl-6-hydrocinnamoyl-α-D-galactopyranosyl)-2-(N-hexacosanoylamino)octadecan-1,3,4-triol (**B**) (51 mg, 0.034 mmol) in THF (2.5 mL) at 0 °C. The solution was stirred at 0 °C until the starting material was consumed (based on TLC). The reaction mixture was diluted with saturated NH<sub>4</sub>Cl (10 mL) and extracted with DCM (2 X 10 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated. Purification by flash column

chromatography on silica gel (petroleum ether/EtOAc 70:30) afforded **C** as an off-white solid (33 mg, 76%): mp 86–88 °C;  $[\alpha]^{21}_D +36.8$  (*c* 1.25, DCM); IR (neat) 3320, 2917, 2850, 1733, 1638, 1046, 695 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.43–7.25 (m, 17H), 7.22–7.19 (m, 3H), 6.28 (d, *J* = 8.4 Hz, 1H), 4.94 (d, *J* = 11.4 Hz, 1H), 4.89 (d, *J* = 7.9 Hz, 1H), 4.88 (d, *J* = 7.4 Hz, 1H), 4.83 (d, *J* = 11.7 Hz, 1H), 4.79 (d, *J* = 11.7 Hz, 1H), 4.71 (d, *J* = 11.6 Hz, 1H), 4.59 (d, *J* = 11.4 Hz, 1H), 4.28–4.24 (m, 1H), 4.15 (dd, *J* = 11.2, 7.0 Hz, 1H), 4.09–4.05 (m, 2H), 3.89–3.78 (m, 5H), 3.60 (br s, 1H), 3.52–3.50 (m, 2H), 2.92 (t, *J* = 7.7 Hz, 2H), 2.59 (t, *J* = 8.0 Hz, 2H), 2.23 (br s, 1H), 2.17 (m, 2H), 1.63–1.59 (m, 4H), 1.28 (m, 68H), 0.90 (m, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 173.1, 172.8, 140.5, 138.4, 138.2, 137.9, 128.7, 128.6, 128.5, 128.5, 128.3, 128.1, 128.0, 127.7, 126.5, 98.9, 79.5, 76.4, 76.1, 74.8, 74.5, 74.2, 73.5, 73.2, 69.7, 69.0, 63.5, 49.4, 37.0, 35.7, 33.6, 32.1, 30.9, 29.9, 29.8, 29.6, 29.6, 26.1, 26.0, 22.9, 14.3; HRMS (ESI) calculated for C<sub>80</sub>H<sub>126</sub>NO<sub>10</sub> (M + H)<sup>+</sup> *m/z*: 1260.9382, found: 1260.9278.

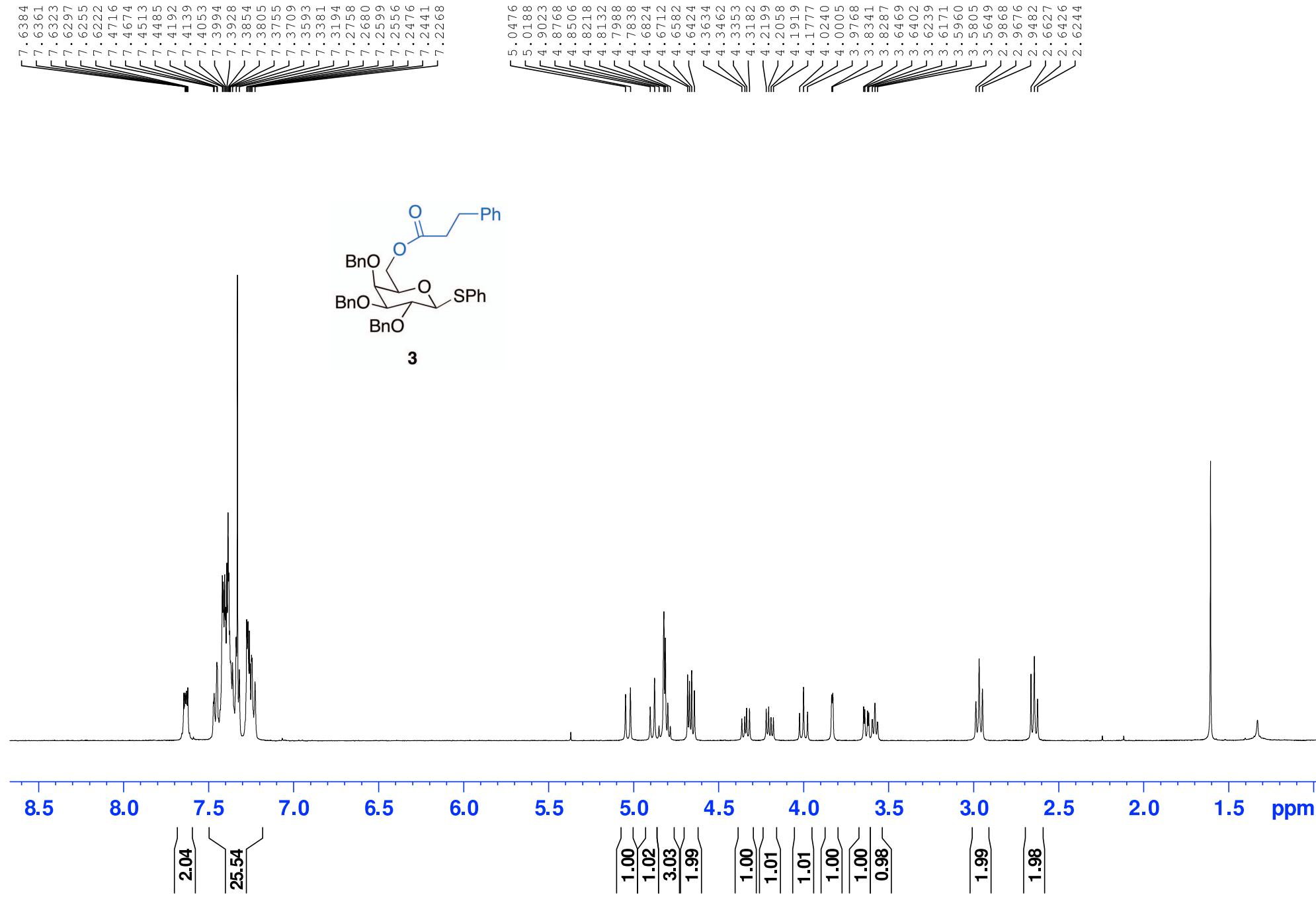
**(2S,3S,4R)-1-O-(6-Hydrocinnamoyl- $\alpha$ -D-galactopyranosyl)-2-(*N*-hexacosanoyl-amino)octadecan-3,4-diol (AH15-1)**

Pd(OH)<sub>2</sub> (20% on carbon, 96 mg) was added to a stirred solution of (2S,3S,4R)-1-O-(2,3,4-tri-O-benzyl-6-hydrocinnamoyl- $\alpha$ -D-galactopyranosyl)-2-(*N*-hexacosanoylamino)-octadecan-1,3,4-triol (**C**) (33 mg, 0.026 mmol) in EtOH (6 mL) and CHCl<sub>3</sub> (2 mL). The mixture was stirred vigorously under H<sub>2</sub> (1 atm) for 12 h. The reaction mixture was filtered through Celite, and the filter cake was washed with 50:50 CHCl<sub>3</sub>/MeOH. The filtrate was concentrated, and the residue was purified by flash column chromatography on silica gel (CHCl<sub>3</sub>/MeOH, 90:10) to afford **AH15-1** as a white solid (22 mg, 90%): mp

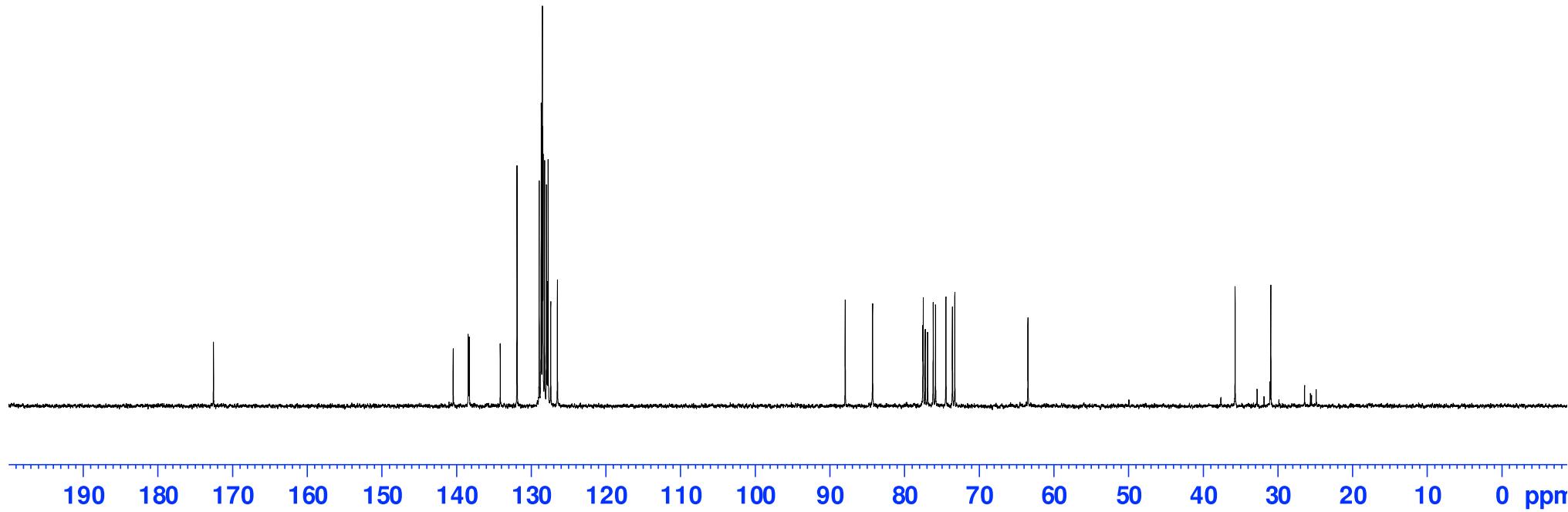
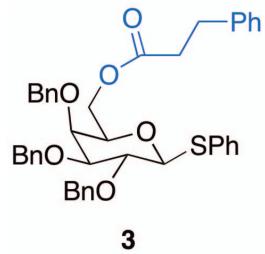
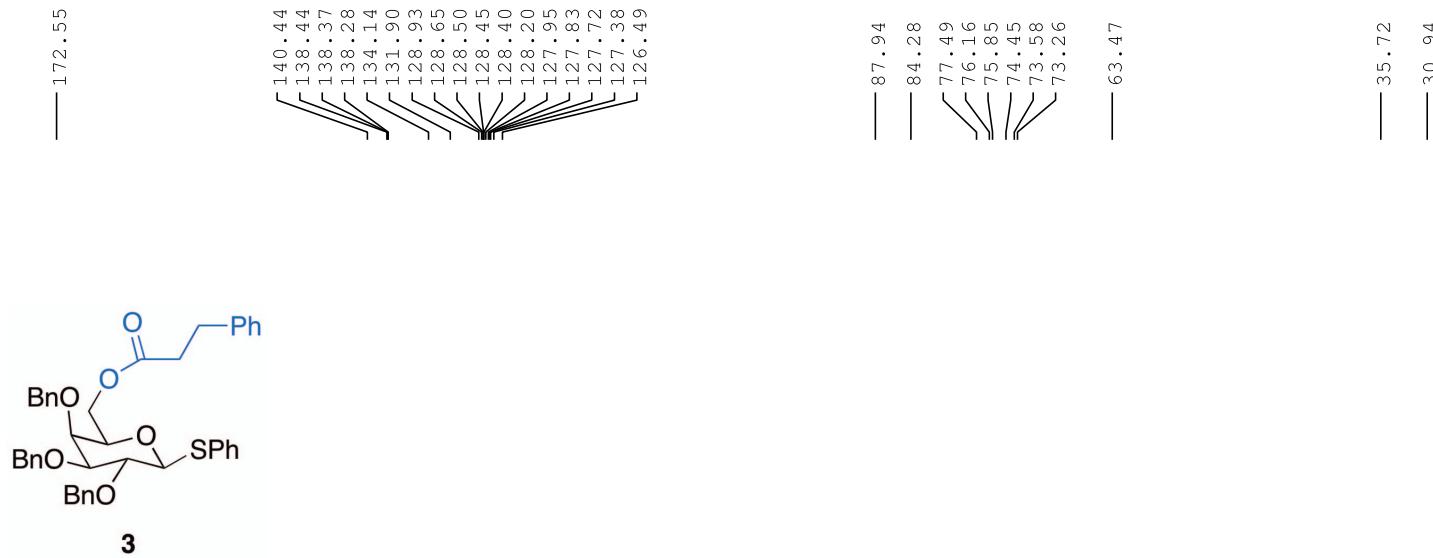
110–112 °C;  $[\alpha]^{21}_D +18.2$  (*c* 0.15, DCM/MeOH, 80:20); IR (neat) 3273, 2959, 2915, 2849, 1725, 1642, 1259, 1074, 1015, 794 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>OD 80:20) δ 7.27–7.23 (m, 2H), 7.18–7.12 (m, 3H), 4.87 (d, *J* = 3.6 Hz, 1H), 4.22–4.20 (m, 2H), 4.17–4.15 (m, 1H), 3.90 (dd, *J* = 6.1, 6.1 Hz, 1H), 3.83 (dd, *J* = 10.6, 4.6 Hz, 1H), 3.79–3.73 (m, 2H), 3.69–3.60 (m, 2H), 3.52–3.46 (m, 2H), 2.91 (t, *J* = 7.7 Hz, 2H), 2.63 (t, *J* = 7.9 Hz, 2H), 2.16 (t, *J* = 7.3 Hz, 2H), 1.65–1.51 (m, 4H), 1.23 (br s, 68 H), 0.84 (t, *J* = 6.4 Hz, 3H), 0.84 (t, *J* = 6.4 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>OD, 80:20) δ 174.1, 173.2, 140.2, 128.4, 128.2, 126.2, 99.5, 74.6, 72.1, 70.0, 69.1, 68.7, 68.4, 67.5, 63.6, 50.0, 36.4, 35.6, 32.5, 31.8, 30.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.3, 29.3, 29.3, 29.3 25.8, 22.6, 13.9; HRMS (ESI) calculated for C<sub>59</sub>H<sub>108</sub>NO<sub>10</sub> (M + H)<sup>+</sup> *m/z*: 990.7973, found: 990.7990.

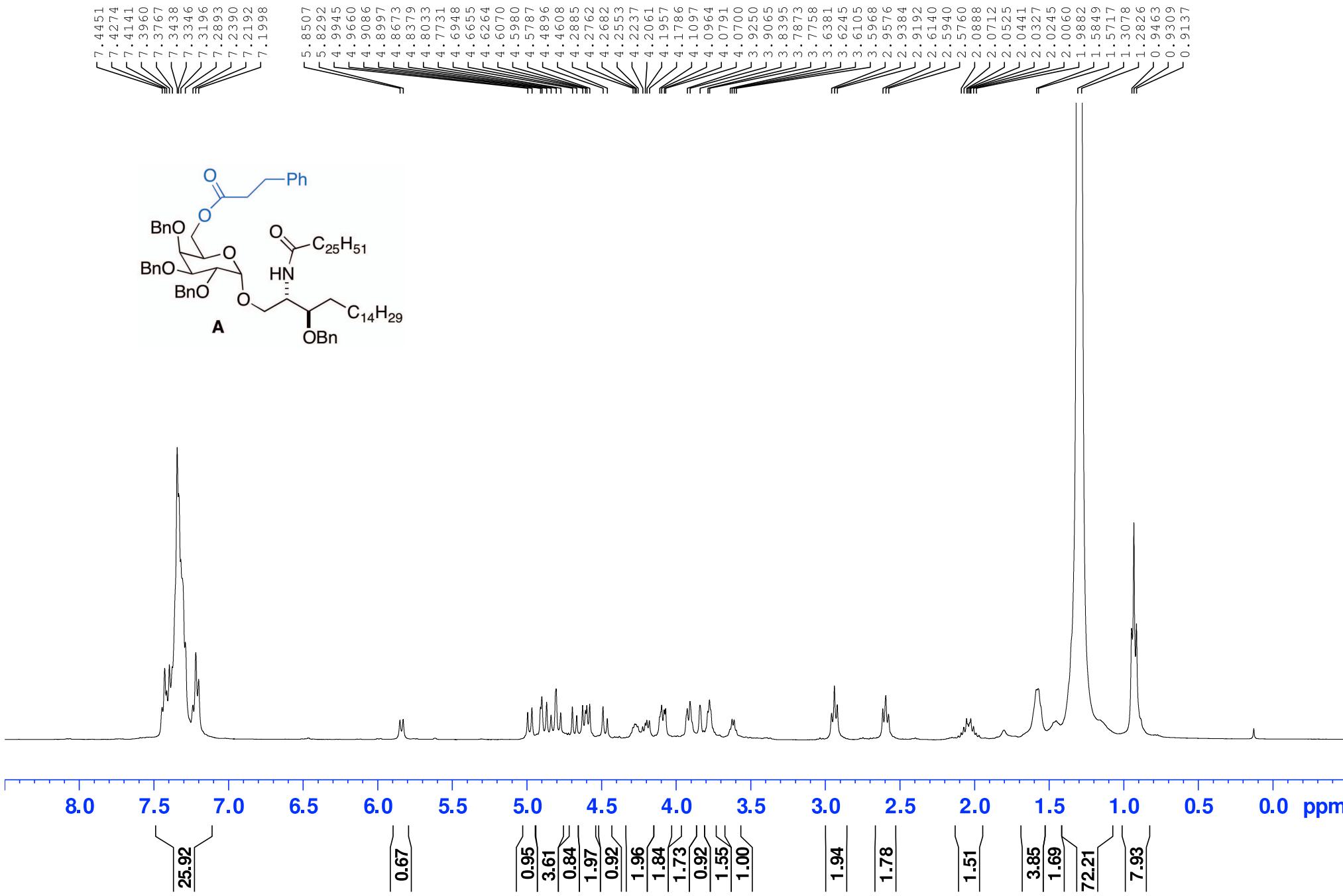
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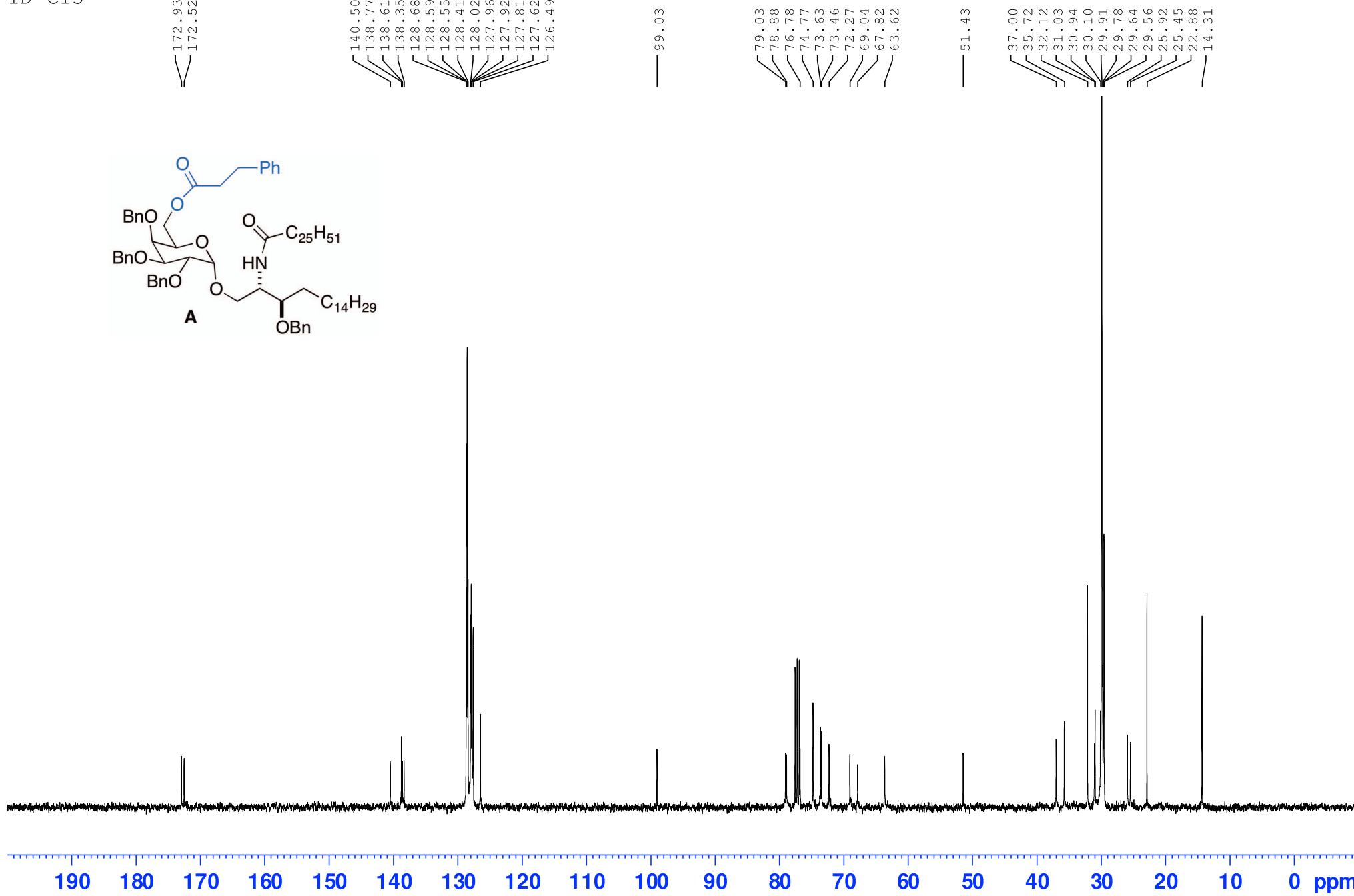


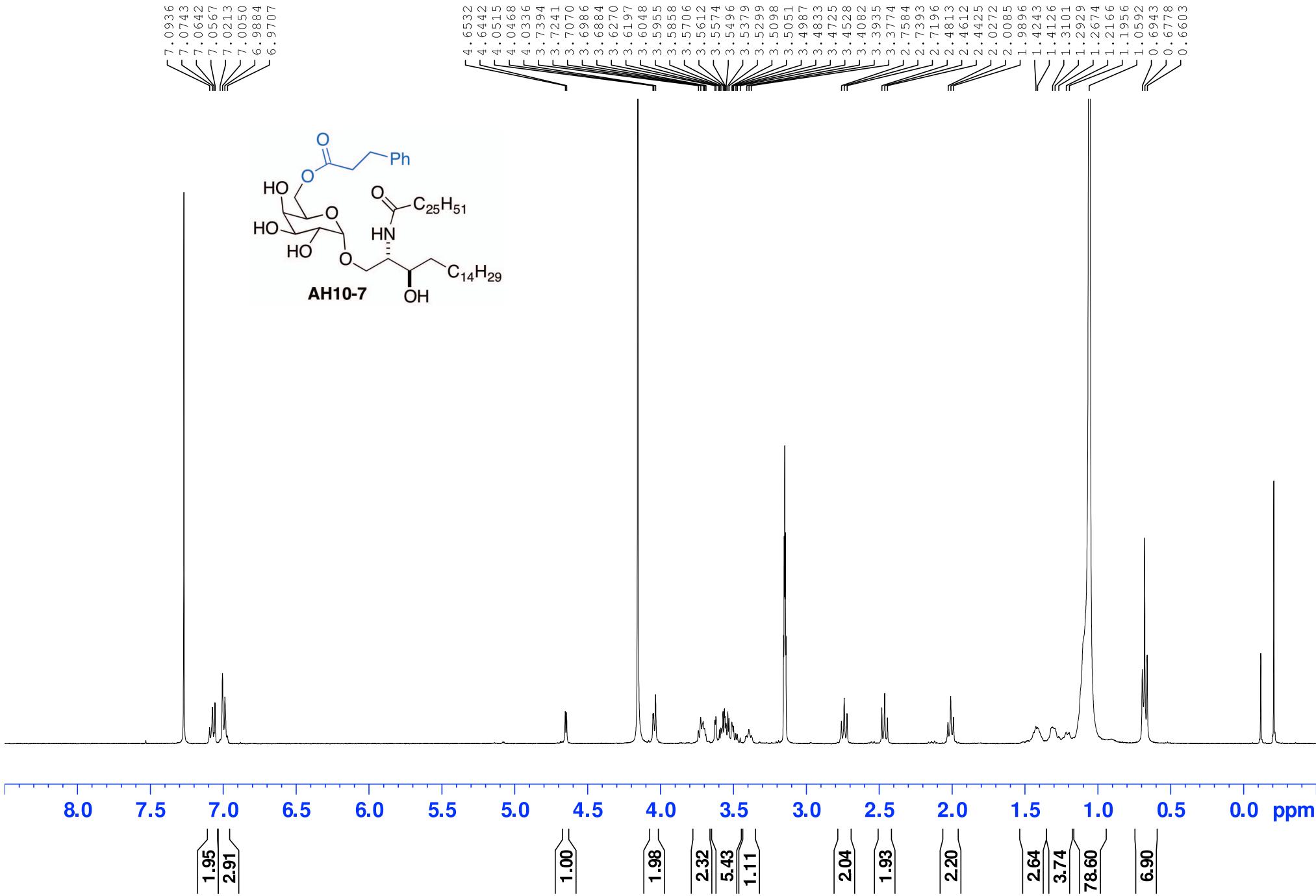
1D C13

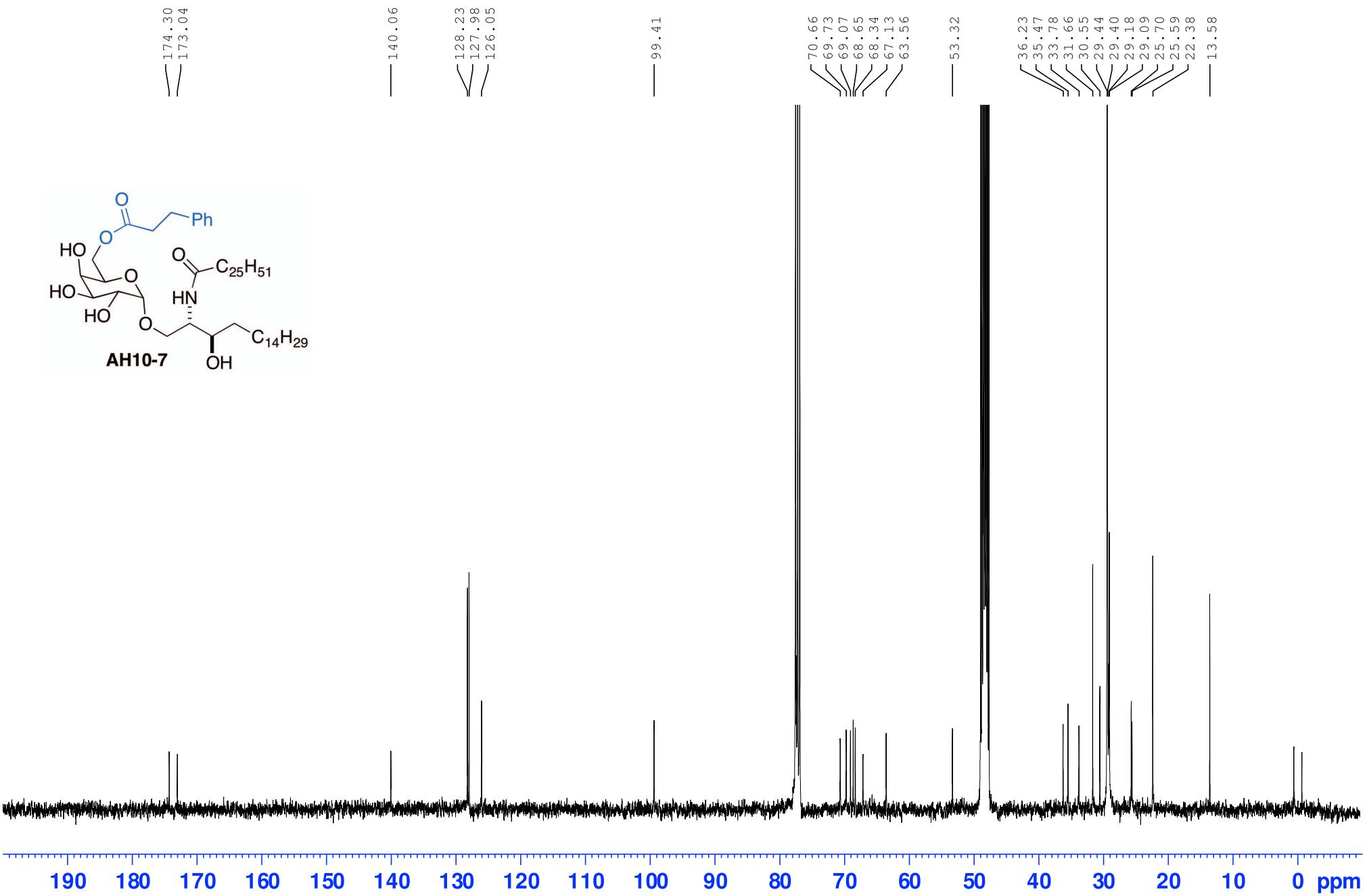
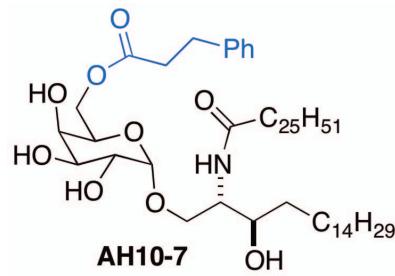


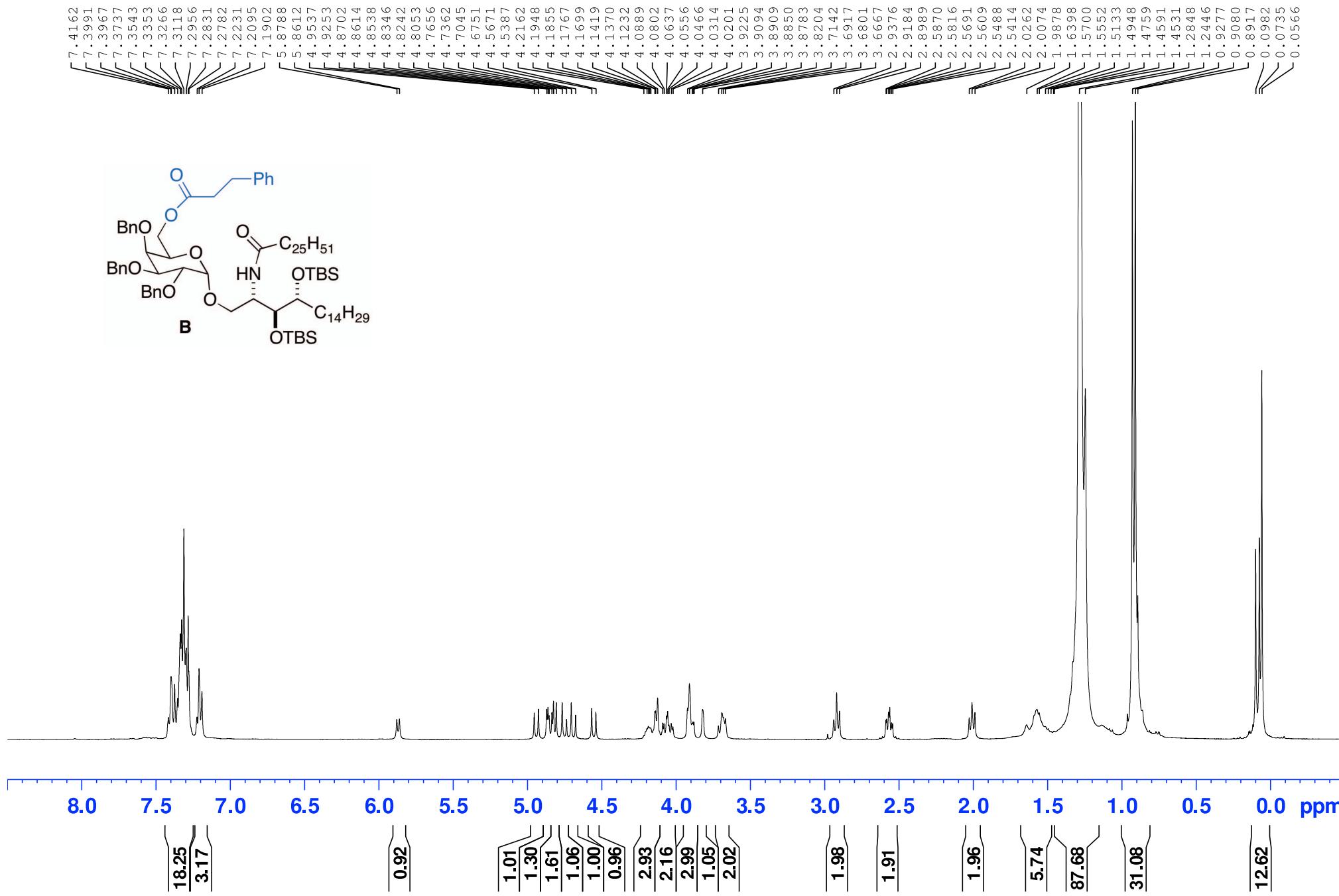


1D C13

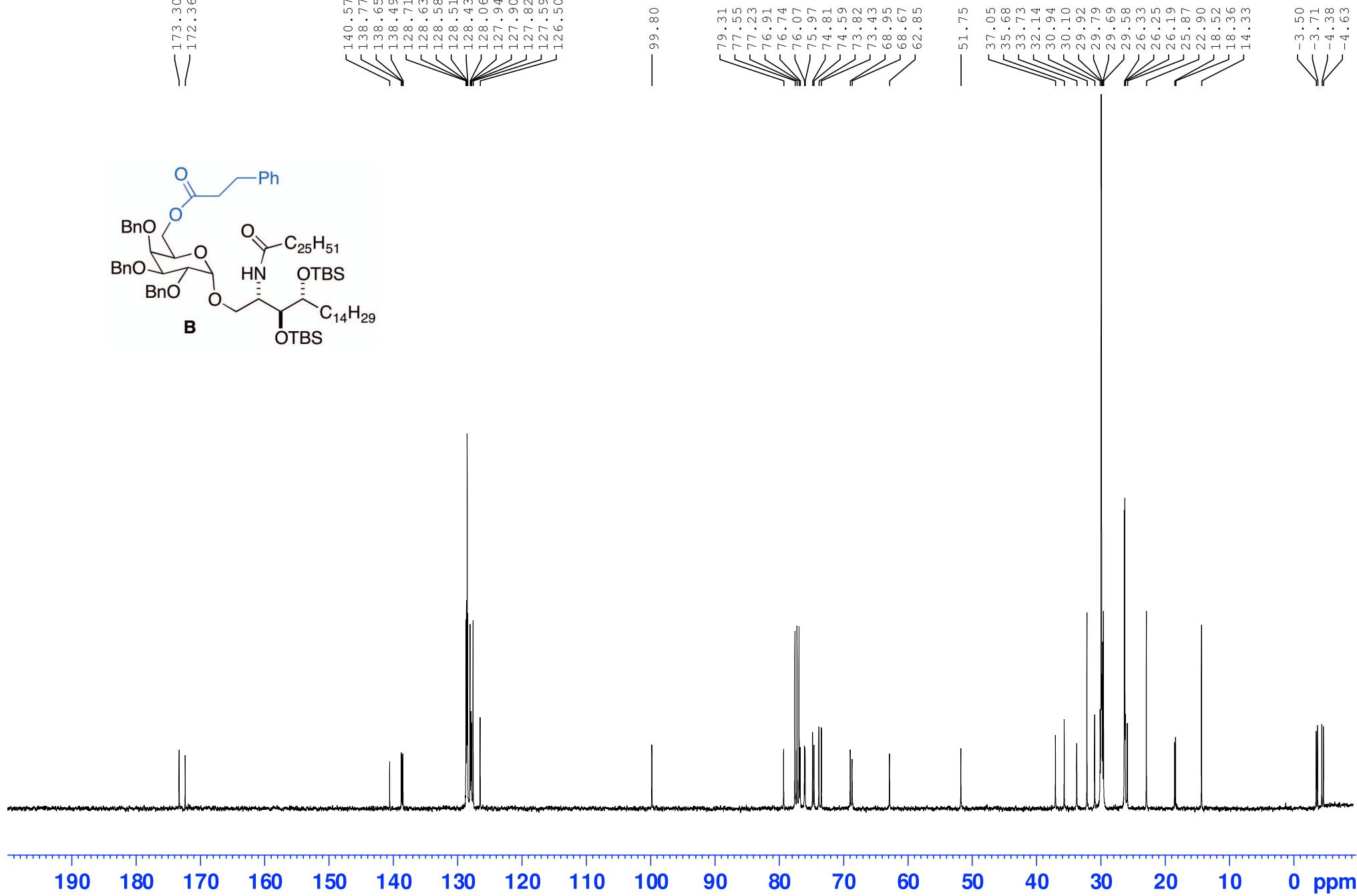




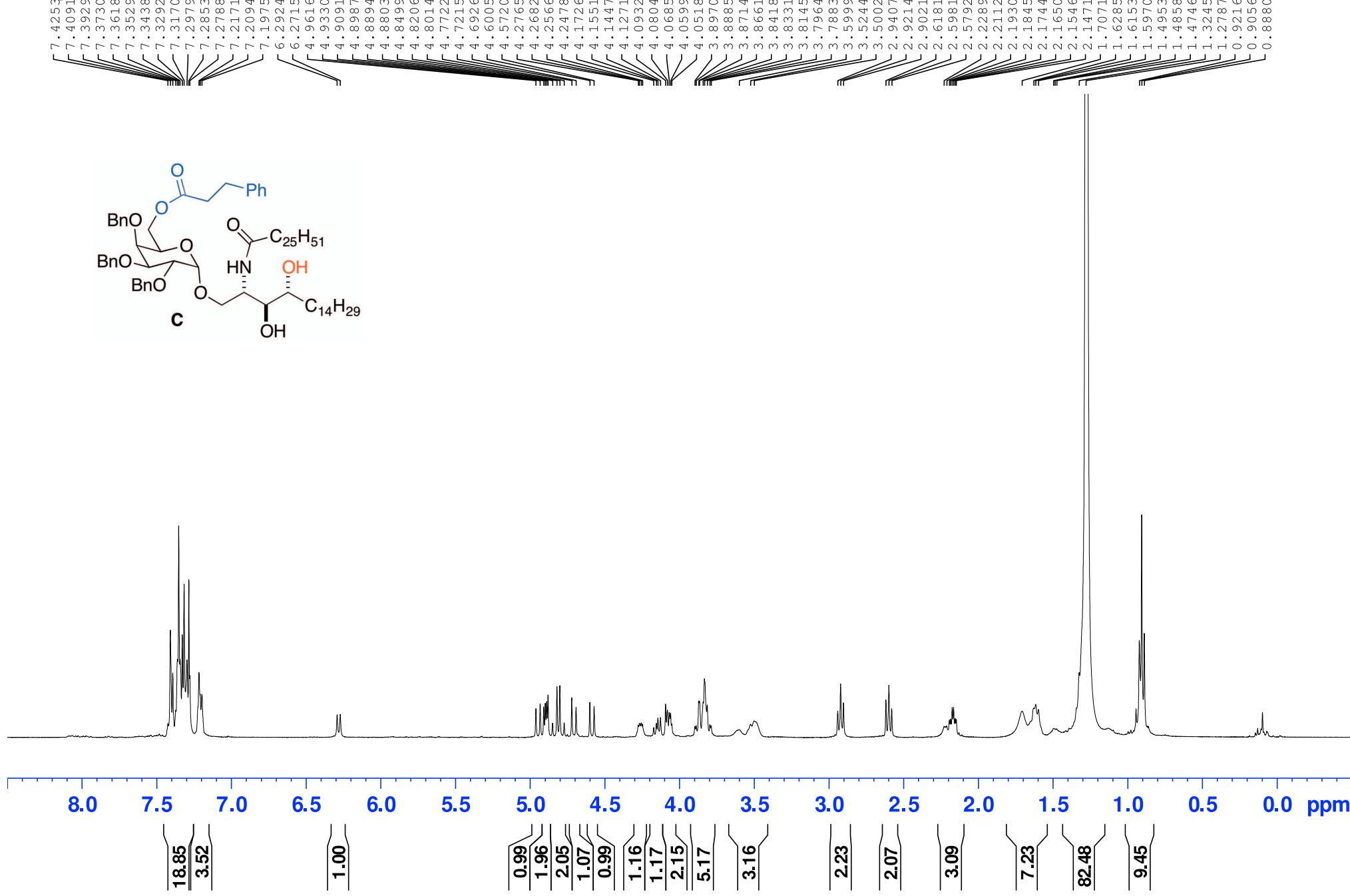




1D C13



<sup>1</sup>H spectrum



1D C13

